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Inhibition of human immunodeficiency virus type 1 transcription and replication by DNA sequence-selective plant lignans

(anti-human immunodeficiency virus/Sp1 site-specific/3′-*O*-methylnordihydroguaiaretic acid)

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ABSTRACT **A plant lignan, 3′-*O*-methyl nordihydroguaiaretic acid (3′-*O*-methyl NDGA, denoted Malachi 4:5–6 or Mal.4; molecular weight 316), was isolated from *Larrea tridentata* and found to be able to inhibit human immunodeficiency virus (HIV) Tat-regulated transactivation *in vivo*, induce protection of lymphoblastoid CEM-SS cells from HIV (strain III_B) killing, and suppress the replication of five HIV-1 strains (WM, MN, VS, JR-CSF, and III_B) in mitogen-stimulated peripheral blood mononuclear cells, all in a dose-dependent manner. Mal.4 inhibits both basal transcription and Tat-regulated transactivation *in vitro*. The target of Mal.4 has been localized to nucleotides −87 to −40 of the HIV long terminal repeat. Mal.4 directly and specifically interferes with the binding of Sp1 to Sp1 sites in the HIV long terminal repeat. By inhibiting proviral expression, Mal.4 may be able to interrupt the life cycles of both wild-type and reverse transcriptase or protease mutant viruses in HIV-infected patients.**

Two major experimental approaches are currently in use for the protection of $CD4^+$ cells from infection with human immunodeficiency virus (HIV) in AIDS patients. One is based on the enhancement of cellular immunity (1, 2), and the other depends on the application of drug therapy. Substantial progress has been made with both approaches. A series of anti-HIV compounds targeting viral replication enzymes and protein processing mechanisms have been characterized (3–6). Each class of compounds is capable of eliminating the replication of most, if not all, wild-type viruses. However, they appear ineffectual in destroying a small pool of coexisting mutant viruses (7, 8) which continue to replicate at a high rate in the presence of these drugs. As a result, the need for rapid CD4 T-cell replacement returns within days following drug treatment (7–9). Thus, controlling the production of the wild-type virus, as well as the mutant virus, becomes an essential consideration for future drug development.

We have recently isolated and characterized a low molecular weight lipophilic lignan, 3-*O*-methyl nordihydroguaiaretic acid (Mal.4) from the perennial shrub creosote bush (*Larrea tridentata*) (10, 11). We report the investigation of Mal.4 and its parent compound nordihydroguaiaretic acid (NDGA) for anti-HIV activity in epithelial cells (COS cells), in mitogen-stimulated peripheral blood mononuclear cells (PBMC), and in cell-free transcription systems. We found that these readily cell-permeable compounds (Fig. 1) were able to suppress HIV replication by blocking the promoter activity of the HIV long terminal repeat (LTR), thereby abolishing both basal transcription and Tat-regulated transactivation. The development of Mal.4-based chemotypes that affect the function of the HIV LTR offers an alternative approach to AIDS therapy.

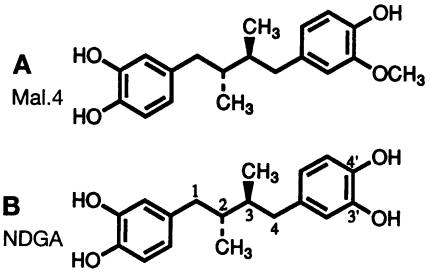

FIG. 1. Molecular structures of Mal.4 and NDGA. (*A*) 1-(3,4-Dihydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-2,3-dimethylbutane (3-*O*-methyl-NDGA; Mal.4) (11). (*B*) 1,4-bis-(3,4-Dihydroxyphenyl)-2,3-dimethyl butane (nordihydroguaiaretic acid; NDGA) (12).

MATERIALS AND METHODS

Tat-Regulated Transactivation Assay and Cell Protection Assay. Transactivation in COS cells and cell protection against viral cytopathic effects in T-lymphoblastoid cells were conducted as described (13, 14).

Effect of Lignans on HIV p24 Antigen Production in Infected PBMC. Stock solutions of the test compounds were prepared in 100% dimethyl sulfoxide (DMSO) at 10 μg/μl and kept at −80°C. Appropriate dilutions of the stock solutions were made in RPMI medium and added to growth medium at a final DMSO concentration of 0.2%. Five HIV-1 strains (VS, WM, JR-CSF, III_B, and MN) were tested in phytohemagglutinin-stimulated PBMC. The production of p24 antigen was measured by enzyme immunoassay (15).

***In Vitro* Transcription Assay.** Each 15-μl *in vitro* transcription reaction mixture contained substrates, transcription buffer, DNA templates (4.15 or 16.6 μg/ml), HeLa cell extract (2.4 mg/ml; 40 μg/reaction), purified Tat protein (3.3 μg/ml), and 20 μCi [α-^{32}P]UTP (400 Ci/mmole; 1 Ci=37 GBq). Following *in vitro* transcription, RNA was purified and analyzed as described (16, 17).

NFκB and Sp1 Gel Mobility-Shift Analysis. Mal.4 at various concentrations was preincubated (15 min; 30°C) with ^{32}P-labeled oligonucleotide probe and then incubated (15 min; 18°C) with purified Sp1 or NFκB. Electrophoresis was performed at room temperature as described (18).

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Abbreviations: HIV, human immunodeficiency virus; LTR, long terminal repeat; DMSO, dimethyl sulfoxide; SV40, simian virus 40; PBMC, peripheral blood mononuclear cells; NDGA, nordihydroguaiaretic acid; Mal.4, 3′-*O*-methyl NDGA; AdML, adenovirus major late; SEAP, secreted alkaline phosphatase.

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RESULTS

Inhibition of HIV Tat-Regulated Transactivation by Mal.4 and NDGA. The basal level of HIV LTR driven SEAP expression in the medium was barely detectable in COS cells, as reported previously (19). Cotransfection of cells with pHIV-LTR-SEAP along with a second plasmid carrying the HIV *tat* gene driven by a cytomegalovirus (CMV) promoter yielded a 60-fold increase in secreted alkaline phosphatase (SEAP) activity in response to Tat stimulation (Fig. 2*A*). This level of transactivation of the HIV LTR by Tat was inhibited by both Mal.4 and NDGA in a dose-dependent fashion when the test compounds were added 12–15 h following cotransfection and SEAP activity was analyzed 48 h later. A nearly superimposable dose–response curve was observed for both Mal.4 and NDGA, with no significant difference in terms of the EC_{50} of these two compounds, which were 25 μM and 20 μM, respectively (Fig. 2*B*).

The Effect of Mal.4 in Infected CEM-SS Cells. A microtiter assay (14) was utilized to quantify the protection conferred by Mal.4 and NDGA from syncytium formation and viral cytopathic effects. The compounds were added to exponentially growing cells just prior to HIV-1 infection [strain III_B; multiplicity of infection (MOI) 0.1; Fig. 3]. Interestingly, Mal.4 exhibited similar potencies for cell protection (EC_{50} = 21 μM; Fig. 3*A*) and inhibition of transactivation in COS cells (EC_{50} = 25 μM, Fig. 2*B*). In addition, Mal.4 induced little cellular toxicity within the range of the concentrations used (Fig. 3*A*). Strikingly, NDGA, which was nearly as effective as Mal.4 in inhibiting transactivation in epithelial cells (Fig. 2), failed to protect CEM-SS cells against viral (III_B) killing (Fig. 3*B*). The two compounds differ by a single methyl group at position 3 of the side chain of the phenyl ring (Fig. 1).

Inhibition of HIV-1 Production in PBMC. The anti-HIV effect of Mal.4 and NDGA was tested on three primary HIV strains (VS, JR-CSF, and WM) and two laboratory HIV strains (MN and III_B) (20). The levels of viral p24 protein in supernatants of both drug-treated and untreated PBMC infected with these HIV strains were measured (Fig. 4). Sharp differences were observed in the inhibitory activities of Mal.4 and NDGA toward these virus strains in cultures of PBMC (Fig. 4*A* and *B*). The dose–response inhibitory effects of Mal.4 and NDGA on strains WM, III_B, and VS showed a very narrow effective concentration range and required high doses to reach >90% inhibition (Fig. 4*A*). For strains III_B and WM, Mal.4 was more effective than NDGA in suppressing virus production. The anti-HIV activities of Mal.4 and NDGA were the same for the VS strain (Fig. 4*A*). The dose–response curves for the JR-CSF and MN strains (Fig. 4*B*) were quite different from those for strains VS, WM, and III_B (Fig. 4*A*). The anti-HIV effect of Mal.4 on these two strains was detected at concentrations as low as 0.06 μg/ml. Mal.4 appeared to be more effective than NDGA in inhibiting replication of viral strains

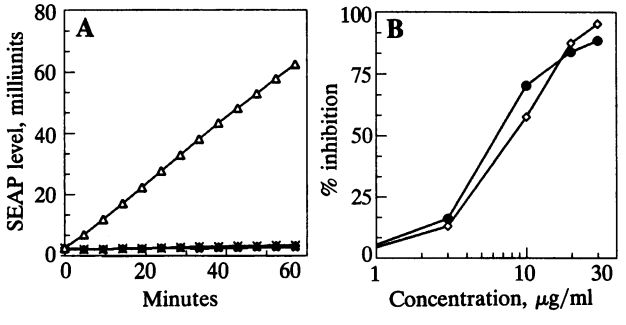

FIG. 2. Tat-regulated transactivation of the promoter activity of HIV LTR (*A*) and dose–response inhibition of transactivation by Mal.4 and NDGA in COS cells (*B*). The inhibitor was added to the cell cultures 12–15 h following cotransfection of pBC12/HIV/SEAP and pBC12/CMV/t_2 as described in ref. 13. Extent of inhibition of SEAP expression was determined at 30 min. X, Control cells without transfection; ★, basal SEAP activity of pBC12/HIV/SEAP-transfected cells. △, SEAP activity following cotransfection with pBC12/HIVLTR-SEAP and pBC12/CMV/t_2. (*B*) Inhibition of Tat-regulated transactivation by Mal.4 (◇) and by NDGA (●). EC_{50} for NDGA, 20 μM; for Mal.4, 25 μM. Percent inhibition was calculated as described (10). Each point represents the average of six determinations from two separate experiments.

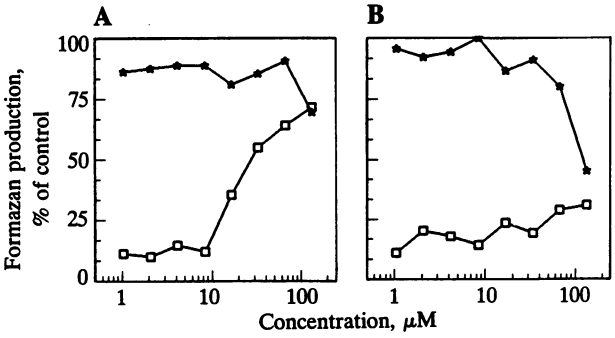

FIG. 3. Quantitation of XTT formazan production (an indicator of cell viability) in CEM-SS cells. (*A*) Mal.4-treated infected (□) and uninfected (★) cells. (*B*) NDGA treated infected (□) and uninfected (★) cells. XTT formazan production for HIV-infected cells without drug protection was 16% of the control (uninfected cells) for *A* and *B*.

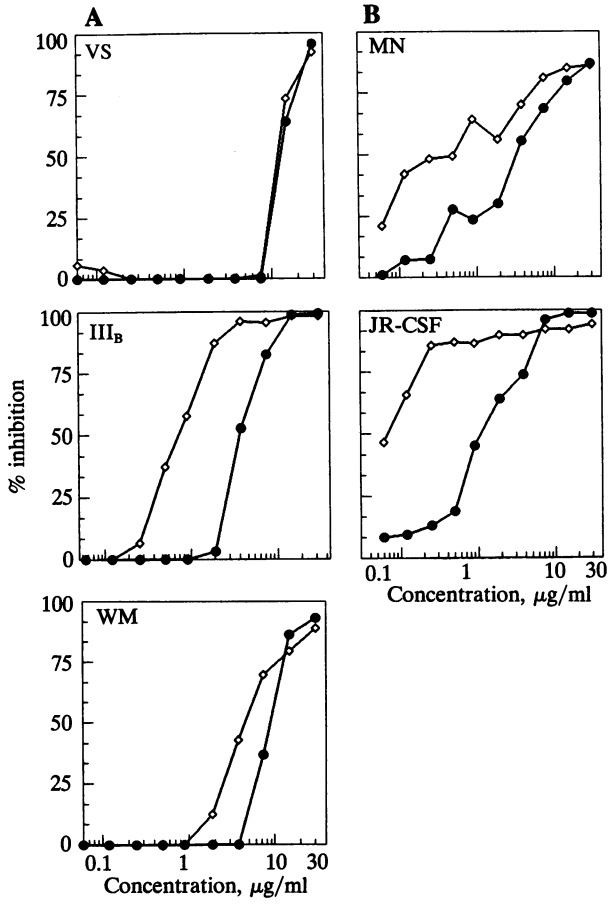

FIG. 4. Inhibition of HIV p24 antigen production in infected PBMC by Mal.4 and NDGA. Inhibition was calculated by comparing p24 levels (pg) from an average of four duplicate cultures of PBMC without drug treatment with p24 (pg) produced from an average of two duplicate cultures of drug-treated and infected PBMC. (*A*) Drug-treated PBMC infected with HIV-1 strains VS, III_B or WM. (*B*) Drug-treated PBMC infected with HIV-1 strains MN or JR-CSF. ◇, Mal.4; ●, NDGA.

JR-CSF and MN. The variation in the dose–responses among HIV strains is probably caused by different growth rates of these viral stains in PBMC.

Inhibition of Promoter Activity of the HIV LTR *in Vitro*. The biochemical mechanism of Mal.4 inhibition of basal and Tat-transactivated HIV transcription was analyzed by using an *in vitro* transcription assay. We have recently shown that the addition of purified Tat protein specifically activates the HIV promoter in HeLa cell extracts (16, 21). The template used in the transcription assays was a plasmid containing the HIV LTR sequence from nt −641 to +78. The plasmid DNA was linearized with the restriction endonuclease *Eco*RI to produce a template with an expected 325-bp run-off transcript. For the Tat transactivation assay, the amount of template added to the reaction was titrated to determine the minimum amount of template required (found here to be 62.5 ng) to give a low, to nondetectable, basal transcription level (Fig. 5*A*, lane 1). Addition of 50 ng of purified Tat resulted in a 9-fold increase in the level of HIV transcription (Fig. 5*A*, lane 2). While no significant difference in transcription was observed with low concentrations of Mal.4 (133 μg/ml) (Fig. 5*A*, lane 3), addition of an optimal concentration (266 μg/ml) of the same compound to the assay resulted in >95% inhibition of Tat-dependent transcriptional activity (Fig. 5*A*, lane 4). The DMSO vehicle alone was not inhibitory (Fig. 5*A*, lanes 5 and 6). In a parallel series of experiments, the effect of Mal.4 on HIV basal transcription was analyzed (Fig. 5*B*). Here, the amount of HIV template was increased to 250 ng so that basal transcription was more readily detectable (Fig. 5*B*, lane 7). Mal.4 efficiently inhibited basal transcription when added at an optimal concentration of 266 μg/ml (Fig. 5*B*, lane 8), a result identical to that obtained with Tat-mediated transcription (Fig. 5*A*, lane 4). Once again, the DMSO control had no effect on basal transcription (Fig. 5*B*, lane 9). Titration curves of the inhibitory effect of Mal.4 on basal and Tat-mediated HIV transcription showed no reproducible difference in sensitivity, suggesting that Mal.4 affects a common regulatory target in transcription.

A series of HIV-template mutations were tested to identify the regulatory site targeted by Mal.4. First, a template containing a deletion of the HIV cis-acting Tat-responsive element (TAR) sequence was analyzed. This template contained HIV LTR sequences from nt −453 to −17. Transcription through the G-free cassette (17, 21, 22) positioned downstream of the HIV promoter results in a transcript of ≈360 bp (Fig. 6*A*, lane 6). Addition of increasing concentrations of Mal.4 to the assay effectively inhibited transcription from the TAR deletion mutant (Fig. 6*A*, lanes 7 and 8). There was no significant difference between the inhibitory effects of Mal.4 on the wild type and this LTR-template mutant (Fig. 6*A*, compares lanes 1–3 and 6–8). These results suggest that Mal.4 does not act at the *TAR* region. Interestingly, the comparative studies of Mal.4 and NDGA (Fig. 6*A*) in the *in vitro* transcription assay revealed that, at the concentrations used here, Mal.4 but not NDGA was an HIV transcriptional inhibitor *in vitro* (Fig. 6*A*, lanes 2 and 3 vs. lanes 4 and 5 and lanes 7 and 8 vs. lanes 9 and 10). Transcription of the template mutant CD38 (23), which contains nt −103 to +80 of the HIV LTR, resulted in a normal level of basal transcription (Fig. 6*B*, lane 1). The addition of 266 μg of Mal.4 per ml to the assay led to a 75% reduction in transcription activity (Fig. 6*B*, lane 2). Transcription of the HIV LTR deletion mutant CD52 (23), which contains sequence from nt −65 to +80, resulted in two obvious changes in transcriptional activity. First, there was a significant decrease in basal transcription (Fig. 6*B*, lanes 1 and 3). In addition, the low basal activity was not further inhibited by the addition of Mal.4 (Fig. 6*B*, lanes 3 and 4). These results beg the conclusion that the essential site for inhibition of HIV transcription by Mal.4 is located within the sequence from nt −103 to −65 of the HIV LTR.

Inhibition of Sp1-Binding Activity in a Gel Mobility-Shift Assay. Two types of primary regulatory sequences have been identified between nt −103 and −65 in the HIV LTR, and include the two NFκB- and one Sp1(3)-binding sites. To determine whether Mal.4 inhibits HIV transcription by preventing the binding of these transcription factors to the cognate sites in this region, gel-shift analyses were performed with ^{32}P-labeled oligonucleotides specific for the Sp1- or NFκB-binding sites. We have previously shown that these stimulated 70Z/3 cell extracts contain high levels of p50–p50 and p50–p65 NFκB-binding activity (24). Incubation of the probe with the 70Z/3 extract resulted in the formation of two gel-shift complexes (Fig. 7*A*, lane 2). The major complex (arrow) is composed of p50–p65, while the faster migrating

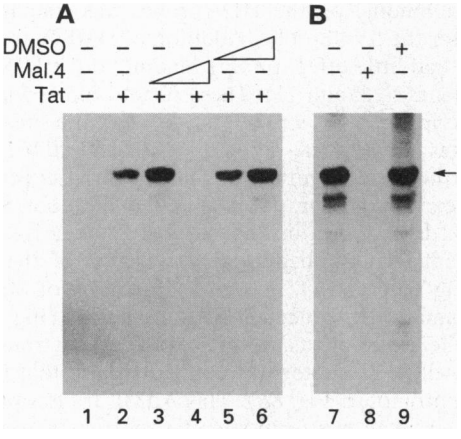

FIG. 5. Effect of Mal.4 on *in vitro* transcription of HIV LTR. (*A*) Template concentration was 4.15 μg/ml. Additions: lane 1, none; lane 2, 50 ng of Tat; lane 3, 50 ng of Tat and Mal.4 (133 μg/ml); lane 4, 50 ng of Tat and Mal.4 (266 μg/ml); lane 5, 50 ng of Tat, volume of DMSO as in lane 3; and lane 6, template plus 50 ng of Tat and a volume of DMSO as in lane 4. (*B*) Template concentration was 16.6 μg/ml. Additions: lane 7, none; lane 8, Mal.4 (266 μg/ml); lane 9, a volume of DMSO as in lane 8. The 325-bp ^{32}P-labeled run-off product is marked by an arrow.

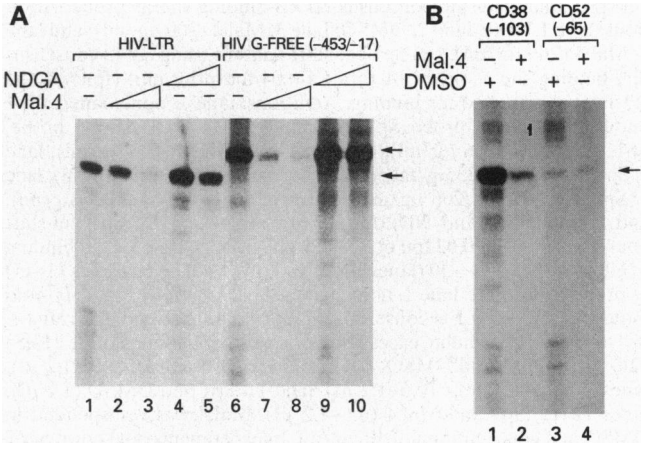

FIG. 6. Effect of Mal.4 and NDGA on *in vitro* transcription of several HIV LTR constructs. (*A*) For lanes 1–5, the HIV LTR template was used. Additions: lane 1, none; lane 2, Mal.4 (233 μg/ml); lane 3, Mal.4 (266 μg/ml); lane 4, NDGA (233 μg/ml); and lane 5, NDGA (266 μg/ml). For lanes 6–10, the HIV G-free template (−453 to −17) was used. Additions: lane 6, none; lane 7, Mal.4 (233 μg/ml); lane 8, Mal.4 (266 μg/ml); lane 9, NDGA (233 μg/ml); and lane 10, NDGA (266 μg/ml). (*B*) Template HIV LTR CD38 (−103 to +80) without (lane 1) or with (lane 2) Mal.4 (233 μg/ml). Template HIV LTR CD52 template (−65 to +80) without (lane 3) or with (lane 4) Mal.4 (233 μg/ml). Concentrations for all HIV LTR templates were 16.6 μg/ml.

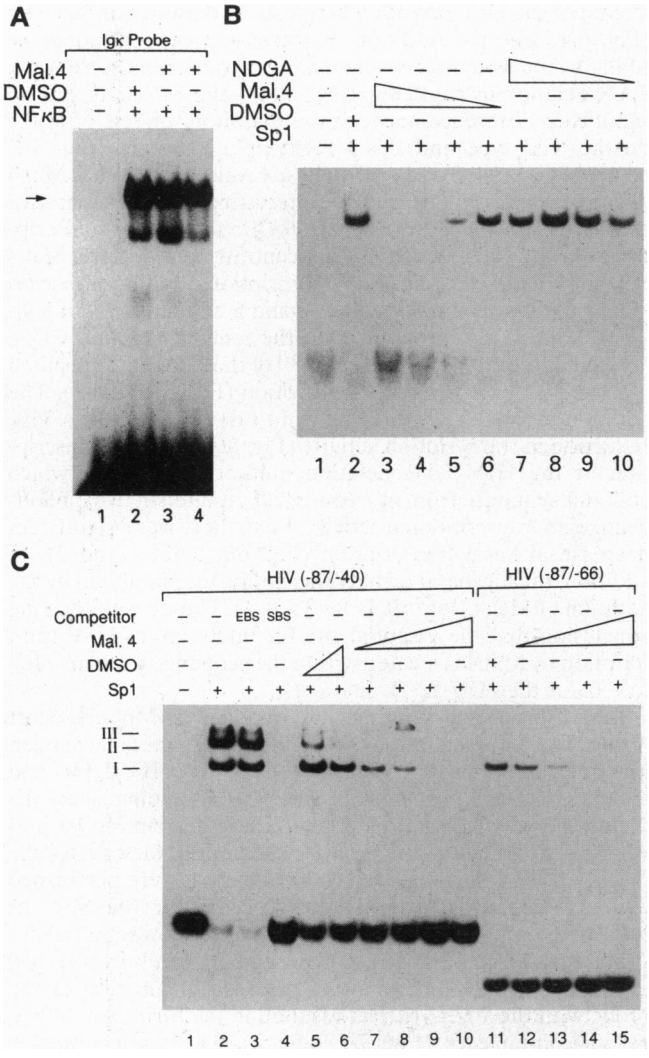

FIG. 7. Mal.4 activity assayed by gel mobility shifts. (*A*) NFκB gel-shift analysis using consensus NFκB-binding site as probe. Additions: lane 1: none; lane 2, DMSO; lane 3, Mal.4 (200 μg/ml); and lane 4, Mal.4 (266 μg/ml). (*B*) Sp1 gel-shift analysis using SV40 consensus Sp1-binding site as probe. A total of 0.04 footprint unit (fpu) of Sp1 (Promega) was used for binding. Additions: lane 1, none; lane 2, Sp1 and DMSO; lane 3, probe, Sp1, and Mal.4 (332 μg/ml); lane 4, probe, Sp1, and Mal.4 (266 μg/ml); lane 5, Sp1 and Mal.4 (200 μg/ml); lane 6, Sp1 and Mal.4 (133 μg/ml); lane 7, Sp1 and NDGA (332 μg/ml); lane 8, Sp1 and NDGA (266 μg/ml); lane 9, Sp1 and NDGA (200 μg/ml); and lane 10, Sp1 and NDGA (133 μg/ml). (*C*) HIV Sp1 gel-shift analysis. A total of 0.02 fpu of Sp1 (Promega) was used for the binding of either HIV −87/−40 (lanes 1–10), or HIV −871/−66 (lanes 11–15) as probe. Additions: lane 1, none; lane 2, Sp1; lane 3, Sp1 and 100-fold molecular excess of Ets consensus oligonucleotide (EBS) (21); lane 4, Sp1 and 100-fold molar excess Sp1 consensus oligonucleotide (SBS) (21); lane 5, Sp1 and DMSO (0.6 μl); lane 6, Sp1 and DMSO (1.2 μl); lanes 7–10, Sp1, Mal.4 (0.6–1.2 μl); lane 11, Sp1 and DMSO (1.2 μl); lanes 12–15, Sp1 and Mal.4 (0.6–1.2 μl). Mal.4 was resuspended in DMSO at a concentration of 10 mg/ml. In experiments not shown here, the inhibition curves obtained with the HIV probes were identical to those obtained with the SV40 Sp1 consensus oligonucleotides. The oligonucleotides used were as follows: Igκ NFκB-binding site, 5′-GATCCAGAGGGGACTTTCCGAGAG-3′, probe in *A*; SV40 Sp1 consensus binding site (SBS), 5′-GATCCCTTGGTGGGGGCGGG-GCCTAAGCTGCGCAT-3′, probe in *B*, competitor in *C*; HIV Sp1-binding sites, 5′-ACTTTCCAGGGAGGCGTGGCCTGGGCGGG ACTGGGGAGTGGCGTCCCT-3′, probes in *C*; and Ets-binding site (EBS), 5′-TCGGGCTCGAGATAAACAGGAAGTGGTC-3′, competitor in *C*.

complex contains p50–p50. Consistent with previous findings, there was specific competition between these bands and the wild-type oligonucleotide but not between these bands and an oligonucleotide containing mutations in the NFκB-binding site (ref. 24 and data not shown). Addition of Mal.4 to the same concentrations which efficiently inhibited HIV transcription (Fig. 6) had no effect on NFκB-binding activity (Fig. 7*A*, lanes 3 and 4).

Similar gel-shift studies were performed with purified Sp1 protein and an oligonucleotide containing the simian virus 40 (SV40) Sp1 consensus binding site (18). Incubation of the oligonucleotide probe with Sp1 results in the formation of a specific gel-shift complex (Fig. 7*B*, lane 2). This complex is specifically affected by competition from an oligonucleotide containing the SV40 Sp1 consensus binding site (18). Preincubation of Mal.4 with this Sp1 oligonucleotide resulted in a dose-dependent decrease in the subsequent Sp1-binding activity (Fig. 7*B*, lanes 3–6) and in Sp1 footprinting (data not shown). Inhibition of Sp1 binding depended upon preincubation of the oligonucleotide probe with Mal.4, suggesting that Mal.4 was not able to dissociate Sp1 once Sp1 is bound to the probe. Interestingly, the concentration of Mal.4 required to inhibit Sp1 binding (200–266 μg/ml) was similar to the concentration required to inhibit transcription from the HIV LTR (Fig. 5). Although inhibition of cytokine-induced HIV transcription via antioxidative processes by NDGA has been recently suggested (25) in our experiments the parent compound NDGA did not affect Sp1-binding activity (Fig. 7*B*, lanes 7–10).

We further tested the ability of Mal.4 to inhibit Sp1 binding to the HIV LTR (Fig. 7*C*). When an oligonucleotide probe that extended from nt −87 to −40 and contained three Sp1-binding sites, Sp1(3), Sp1(2), and Sp1(1), was used, specific gel-shift complexes I, II, III were formed in the presence of Sp1 (Fig. 7*C*, lane 2). These complexes, while not affected by competition by the EBS oligonucleotide (Fig. 7*C*, lane 3), were absent in the presence of SBS competitor (Fig. 7*C*, lane 4). With an oligonucleotide probe extending from nt −87 to −66 and containing one Sp1-binding site Sp1(3), only one Sp1 complex (I) was observed (Fig. 7*C*, lane 11). Preincubation of the oligonucleotide probes with increasing amounts of Mal.4 inhibited the formation of the Sp1–DNA complexes (Fig. 7*C*, lanes 7–10 and 12–15). The concentration of Mal.4 required to inhibit Sp1 binding on the HIV probes was similar to that observed for the SV40 probe. Addition of DMSO, the vehicle for Mal.4, had little effect on Sp1 binding to the HIV probes (Fig. 7*C*, lanes 5, 6, and 11). These results, along with the *in vitro* transcription assay, strongly suggest that the site of action of Mal.4 lies between nt −87 and −40 of the HIV LTR.

As a further test, the effect of Mal.4 on transcription from the SV40 early promoter, which is dependent upon Sp1 (26), was analyzed. As shown in Fig. 8*A*, the SV40 early promoter was efficiently transcribed in the presence of the control DMSO (Fig. 8*A*, lane 1). In contrast, addition of Mal.4 (266 μg/ml) dramatically reduced SV40 transcription (Fig. 8*A*, lane 2). In parallel experiments, the effect of Mal.4 on transcription from the AdML promoter was compared with that from the HIV LTR promoter (Fig. 8*B*). This AdML transcription unit is controlled by an upstream stimulatory transcription factor (USF) binding site (27). Addition of Mal.4 (200 μg/ml) to an *in vitro* transcription reaction containing the AdML promoter resulted in less than a 50% drop in transcription activity. However, the same concentration of Mal.4 inhibited HIV transcription and Tat transactivation by greater than 90%. These results suggest that Mal.4 preferentially inhibits transcription from promoters activated by the Sp1 transcription factor.

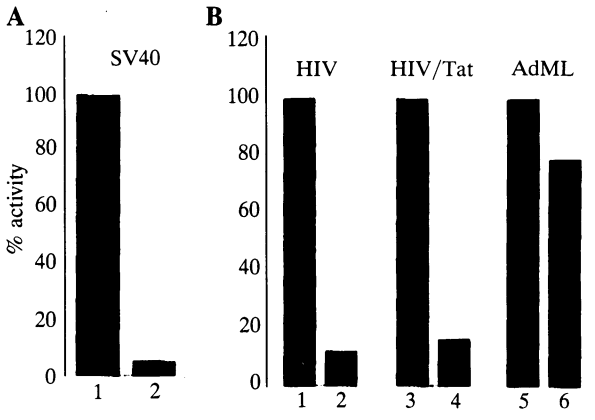

FIG. 8. Mal.4 effects on *in vitro* transcription of different viral promoters. Reactions were carried out as described in the legend to Fig. 5. pSV2CAT template (8.3 μg/ml) without (lane 1) or with (lane 2), Mal.4 (266 μg/ml). (*B*) HIV LTR template (16.6 μg/ml) without (lane 1) or with (lane 2) Mal.4 (200 μg/ml). HIV LTR template (4.15 μg/ml), and Tat (3.3 μg/ml) without (lane 3) or with (lane 4) Mal.4 (200 μg/ml). Adenovirus major late (AdML) template (16.6 μg/ml) without (lane 5) or with (lane 6) Mal.4 (200 μg/ml). Quantitation of ^{32}P-labeled RNA electropherograms was done on the PhosphorImager. The ^{32}P cpm observed with the template in the absence of Mal.4 was set as 100%.

DISCUSSION

Control of Replication by Specific Transcription Inhibitors. In this study, we have reported that Mal.4 inhibits HIV replication, in part, by affecting HIV LTR transcription. The DNA sequence critical for the Mal.4 inhibition of transcription contains two NFκB- and one Sp1-binding sites (nt −103 to −65). Gel mobility-shift studies have further shown that Mal.4 does not affect NFκB binding but does strongly inhibit Sp1 binding. Previous studies of the HIV-1 promoter have demonstrated that basal transcription and Tat-mediated transactivation require the cooperative activity of several promoter-bound cellular transcription factors (28).

It is unclear how Mal.4 interferes with Sp1 binding to the HIV LTR promoter. The compound may act by directly binding to the Sp1 protein and/or Sp1 consensus sequence and thereby behaving like a general transcription inhibitor. Alternatively, the effect of Mal.4 may be more indirect. This agent may interact with DNA sequences in the vicinity of the Sp1 sites in the HIV LTR (and SV40), selectively inducing a local structural distortion of DNA. In this case, transcription could be inhibited by the creation of a DNA structure unfavorable for Sp1 protein binding. Consistant with this view is the recent finding that structural distortion of DNA induced by the drug (+)-CC-1065 was likely responsible for the inhibition of binding of Sp1 to 21-bp repeats of SV40 DNA (29). Similar studies with Mal.4 and other related lignans on the topology of HIV LTR are underway to provide a better understanding of the mode of action of these plant-derived agents.

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